REMARKS

The application referenced above was filed to continue prosecution of the claims pending in U.S.S.N. 09/286,268. The remarks presented here address the outstanding grounds for rejection (those in the final Office Action mailed November 3, 2000 and the non-final Office Action mailed August 22, 2001) and, in particular, provide information requested by Examiner Unger and her Supervisor, Examiner Caputa, in the interview conducted at the U.S. Patent and Trademark Office on February 21, 2002 (herein, "the interview"). Should the present submission be non-persuasive in any way, the Examiners (both Examiner Unger and Examiner Caputa, together) are kindly asked to telephone the undersigned.

Claims 8 to 12 are pending in the application. Claim 8 has been amended, as discussed during the interview, to specify that the cell growth is a "malignant" growth. Further, the claim has been amended to insert the phrase "at risk for malignant cell growth." The amendment is supported by original claims 6 (now canceled) and 8. The description of Fig. 4 (on page 5, beginning at line 18) has also been amended to incorporate a description of the conditions represented by each of the bars of the bar graph. This description appears in the specification as filed at page 19, lines 13-19. No new matter has been added.

35 U.S.C. § 112, ¶ 1

The primary issue in this case is whether Applicants' specification satisfies the statutory requirement for "enablement."

The examiner argues that claim 8 reads on inhibiting malignant cell growth in a mammal at risk for neoplastic cell growth (Office Action dated August 22, 2001, at page 3), and Applicants agree. The claim covers methods in which mammals "at risk" are treated with nucleosomes that elicit antinuclear autoantibodies (ANAs) and thereby protect them from such growth (by, for example, reducing the likelihood that a growth will develop). Accordingly, Applicants have amended claim 8 to recite "a method of inhibiting malignant cell growth in a mammal at risk for malignant cell growth." In the remarks that follow, Applicants address enablement rejections put forth by the Examiner in the Office Actions dated March 30, 2000 and November 3, 2000, as well as those in the non-final Office Action dated August 22, 2001.

The Examiner has argued (Office Action mailed March 30, 2000):

> One cannot extrapolate the teaching of the specification to the enablement of the claims because the claims are drawn to the inhibition, which reads on prevention, of neoplastic cell growth in mammals at risk of developing said growth. Although the specification defines individuals at risk, as disclosed above, there is no teaching in the specification as to when the method is to be initiated other than that the prophylactic treatment can be begun before there is any evidence of a tumor. Certainly the majority of the population of the United States has been inadvertently exposed to carcinogenic substances through exposure, for example, to second hand smoke and all of the population has been exposed to nuclear radiation from the sun.

The Examiner then concluded (Office Action mailed March 30, 2000):

> The specification does not provide either guidance on or exemplification of how to determine which of these "individuals at risk" would be candidates for the method.

This rejection was discussed during the interview. At that time, the Examiners advised Applicants to outline the arguments made previously and during the interview. Applicants understood that these arguments would be sufficient to overcome the rejection.

The arguments of record establish that, although the process of deciding who is "at risk" for malignant cell growth is not an exact science, it is an endeavor routinely and necessarily undertaken by physicians in consult with one another and their patients. In support of that position, Applicants submitted a number of references that described some of the ways physicians recognize and plan treatment for patients "at risk" for malignant cell growth. These studies focused on (i) patients with a family history of cancer (*e.g.*, breast and colon cancer); (ii) patients displaying genetic markers for cancer (*e.g.*, BRCA and BRCA2 signal risk of breast cancer); (iii) patients whose tumors have been treated with chemotherapy or surgery; and (iv) patients exposed to carcinogens (*e.g.*, chemicals and pollutants such as those in tobacco and radiation, including ultraviolet radiation). If an individual is within these, or any other populations known to be "at risk," they are candidates for the method now claimed. These patients and their physicians can determine whether they will be treated with nucleosomes, just as all patients and physicians reach conclusions about any other available therapy. One of ordinary skill in the art would not have to resort to undue experimentation to determine whether a patient is "at risk." According, this ground for rejection should be withdrawn.

When claim 8 was amended previously, the Examiner reasoned that it covered a method of inhibiting malignant neoplastic cell growth in a mammal that bears a tumor burden (Office Action dated August 22, 2001). Because claim 8 now recites "a method of inhibiting malignant cell growth in a mammal at risk for malignant cell growth," this ground for rejection is moot.

The Examiner's prior remarks also show considerable concern about dosage (see, in particular, the Office Action dated March 30, 2000). In addition to the remarks of record, Applicants emphasize here that: (1) the presence of ANAs in certain populations in no way makes dosing unpredictable; (2) treatments with other antigens have been successfully extrapolated from murine models to clinical application and there is no reason to expect that one could not do the same here; and (3) ANAs only specifically bind nuclear material. The third showing is prompted by Banks *et al.*, Jacob *et al.*, and Raz *et al.* (see, *e.g.*, the Office Actions dated March 30, 2000 and November 3, 2000). The reference the Examiner continued to cite during the interview was Banks *et al.*, which examined ANAs in the context of massive cell death. The Examiner argued that non-specific binding would sequester ANAs and thereby make dosing unpredictable.

(1) During the interview, the Examiner noted that ANAs, which are generated following administration of nucleosomes, are naturally present in at least some people. The Examiner questioned why, then, were those antibodies ineffective against malignant cell growth; if Applicants propose to treat patients at risk for malignancies, and ANAs are already present in the general population, why do cancers still develop?

It is true that ANAs are present in some people (*e.g.*, it is not unusual to find ANAs in the elderly), and there have been a number of studies in which ANAs were characterized in some manner in patients who have cancer. However, the fact that ANAs are present, even in cancer patients, in no way forces one of ordinary skill in the art into undue experimentation. To the contrary, the studies concerning ANAs and the conventional drug development protocols known at the time the present application was filed, will guide those who wish to determine an appropriate dosage of nucleosomes for humans. If the levels of ANAs typically seen in, for example, elderly people are not sufficient to prevent cancer, one would reasonably expect that higher levels must be generated in the method now claimed.

Applicants have attached a publication to illustrate the type of information available about ANA levels in cancer patients. In the publication attached, Fernandez-Madrid *et al.* (*Clin. Cancer Res.* 5:1393-1400, 1999; herein, "Fernandez-Madrid," a copy of which is attached at **Tab A**) found that ANAs were more common in patients with "biopsy-proven, locally advanced or metastatic lung cancer" (page 1393, column 2) than in "subjects without a history of cancer" (page 1394, column 1). Fernandez-Madrid states (page 1394, column 2):

> Although 67% of the cancer sera antibodies showed reactivity even at 1:2000 dilution, only one normal serum showed a titer greater than 1:500 using these criteria.

Fernandez-Madrid comments on the reactivity of ANAs in cancer patients relative to that seen in the elderly ("[a]lthough autoantibodies are more prevalent in the elderly population (52), they are usually of low titer, unlike those reported here" (page 1398, column 1)). He also speculates as to the stimulus for antibody development in the cancer patients: "[i]t is conceivable that ANA development in heavy smokers may be an early indication of DNA damage in susceptible subjects" (page 1398, column 1). Perhaps most significantly, Fernandez-Madrid finds some correlation between ANAs and PFS (progression-free survival). He states, "[s]ome of these antibodies were associated with a prolonged survival without disease progression" (abstract). Thus, there is at least some indication that naturally occurring levels of ANAs provide some protection against disease progression (which may be especially significant in the context of the present invention, which encompasses treatment of patients who have had a tumor removed by conventional methods. Nothing that was known about ANAs or their level of expression in various populations would force one of ordinary skill in the art to resort to undue experimentation. Empirical studies to determine effective doses are done routinely when bringing a therapeutic agent from laboratory studies to human clinical trials and, finally, to the clinic. The amount of experimentation that may be required here is no more than what is usually required in the course of drug development.

(2) Following from the reasoning above, and as discussed during the interview, Applicants' specification is enabling even though it does not provide a dose of nucleosomes shown to be effective in human patients. Human data is not required, and it is well within the ability of one of ordinary skill in the art to determine an appropriate dosage, particularly given an

animal model. Applicants have argued that essentially every FDA-approved agent on the market today has traveled a similar path; as described above, it is typical to test an agent first in a pre-clinical model (*e.g.*, an *in vitro* assay or cell culture) and to employ at least one animal model before moving through the standard phases of testing for safety and efficacy in humans. There is really no alternative; for many reasons (practical, financial, ethical, and others), pharmaceutical companies simply do not administer agents to humans without substantial prior testing. It should be evident that those of ordinary skill in the art have extrapolated successfully from murine models to clinical application with other therapeutic agents, including other antigens (if they had done so with the present antigen, the invention would have no novelty). As an example, during the interview, we discussed antigens administered to stimulate protective responses against an influenza virus. "Flu" vaccines and others types of vaccines are especially relevant to the method claimed here because in both instances, the antigens administered can be administered prophylactically. Those of ordinary skill in the art successfully extrapolate from animal models to human application and there is no reason to expect that one could not do the same here (the Examiner's concern that ANAs may bind non-nuclear material is discussed below).

(3) The third and final point in response to the Examiner's concern about dosage is that ANAs only react with nuclear material. Prior to the interview, the Examiner had not been satisfied that Applicants had addressed this issue. The reference by Banks *et al.* was the only reference raised during the interview, but the same issue is raised by Jacob and Raz (do ANAs cross-react with non-nuclear material and, if so, would that cause one of ordinary skill in the art to resort to undue experimentation to practice the method Applicants now claim). Banks *et al.* examined ANAs in the context of massive cell death, where components of the nucleus, including histones, are disrupted and may become associated with other parts of cells, such as their outer membranes. While Banks and others may have concluded that there was some cross-reactivity (*e.g.*, Raz *et al.* begin their discussion by stating that they have "demonstrated a direct and specific cross-reaction between SLE anti-DNA autoantibodies and membrane proteins, present on the surface of cells from different tissues, short-term cultures and permanent lines"), there is contradictory evidence that ANAs *are* histone-specific.

In support of this view, Applicants submit an abstract from Van Bruggen *et al.* (*Ann. Med. Interne. (Paris)* 147:485-489, 1996; **Tab B**). The abstract reviews some of the "classical"

concepts about the relationship between antibodies that bind double-stranded DNA (dsDNA) and autoimmunity. Van Bruggen refers to the possibility of "cross-reactivity of anti-dsDNA with glomerular constituents like heparan sulfate (HS) and laminin," and then states (emphasis added):

> However, subsequent research has indicated that this *cross-reactivity is due to nucleosomal antigens* (histones and DNA) *complexed to the auto-antibodies*. The cationic histone part of the complex is responsible for the binding to the anionic HS [heparin sulfate]. This binding also occurs in vivo since renal perfusion of nucleosome complexed antibodies leads to abundant binding of auto-antibodies to the GBM, while enzymatic removal of HS from the GBM, decreases this binding considerably. *Non-complexed antibodies did not bind at all.*

Thus, the idea, while once popular, that ANAs demonstrate substantial cross-reactivity with non-nuclear components of cells is no longer supported and provides no basis for the present rejection. Moreover, even if there were some degree of non-specific binding, that does not mean that dosing is unpredictable or that one of ordinary skill in the art would have to resort to undue experimentation to determine an effective dose. Accordingly, Applicants request that rejection for lack of enablement be withdrawn.

The Examiner also rejected claims 8 and 10 to 12 because the specification allegedly does not provide enablement for a method of inhibiting malignant neoplastic cell growth in a mammal by administering bacterial nucleosomes. The Examiner states (Office Action dated August 22, 2001 at page 10):

> [o]ne cannot extrapolate the teaching of the specification to the scope of the claims because the invention is specifically drawn to the discovery that antinuclear autoantibodies specifically bind nucleosomes that are present on the surface of tumor cells. Although the response against bacterial DNA may be greater than the response to mammalian DNA, that response would be expected to be to epitopes not found on the mammalian DNA, that is not seen as self. Since the autoantibodies produced are required to bind to mammalian tumor cells which express mammalian ANA epitopes on the surface of the cells, it could not be predicted, nor would it be expected that autoantibodies produced against an antigen (for example hypomethylated CpG) which is not characteristic of the mammalian antigen would bind to the mammalian tumor cells.

But, even if some antibodies raised against bacterial nucleosomes bind only nucleosomes that include hypomethylated CpG-containing DNA (as the Examiner suggests; see above), there

is no evidence that such antibodies would arise to the exclusion of antibodies able to bind immunogenic epitopes on both bacterial and mammalian nucleosomes. In fact, Applicants have demonstrated that antibodies raised against mammalian nucleosomes are able to bind nucleosomes that include bacterial DNA. The monoclonal antibody ANA 2C5 binds reconstituted nucleosomes consisting of histones and bacterial DNA (specification at page 8, line 17 to page 9, line 16). Thus, the immunogenic epitope that leads to the generation of ANA 2C5 is present on both mammalian and bacterial nucleosomes, and there is no reason to expect that undue experimentation would be required. Accordingly, this ground for rejection should be withdrawn.

The Examiner also rejected claim 11 because the specification allegedly fails to enable methods in which nucleosomes are encapsulated in liposomes. The Examiner states (Office Action dated August 22, 2001 at page 11):

> One cannot extrapolate the teaching of the specification to the scope of the claims because the encapsulation of the nucleosome within the liposome would be expected to protect the nucleosome from coming into contact with the elements of the immune system required to produce the claimed autoantibodies.

The Examiner cites Gregoriadis and Florence (*Drugs* 45(1): 15-28 (1993)) to support the proposition (at page 12) that:

> [I]t cannot be predicted that sufficient nucleosomes will be presented to the immune system to produce sufficient antinuclear autoantibodies to inhibit malignant neoplastic cell growth as claimed.

Applicants respectfully traverse this ground for rejection. One of ordinary skill in the art would not expect liposome-encapsulated nucleosomes to be protected from the immune system. To the contrary, it was known in the art at the time the application was filed that, following administration to a patient, liposome-encapsulated antigens undergo intracellular uptake, followed by presentation to the immune system by antigen-presenting cells. Even the reference cited (Gregoriadis and Florence) contradicts the proposition that liposomes protect antigens

stating (at page 21, column 2, to page 22, column 1, emphasis added):

> It has been nearly 20 years since liposomes were first shown to improve immune responses to entrapped diphtheria toxoid (Allison & Gregoriadis 1974). Liposomes are now one of the few major nontoxic candidate adjuvants for human use currently under investigation with a wide range of bacterial, viral, protozoan, tumour, and other antigens. In immunization experiments, *a variety of liposomal antigens administered parenterally or enterally were effective in protecting animals against disease*[.]

In addition, Applicants provide a patent with claims covering methods of treating cancer by administering liposome-encapsulated antigens (**Tab C**; U.S. Patent No. 5,738,867, filed June 6, 1995 and issued April 14, 1998). Claim 1 covers an antitumor vaccine that includes a GA733-2 antigen encapsulated in, or covalently bound to, a liposome carrier.

Thus, a skilled practitioner would not expect liposome-encapsulated nucleosomes to be protected from the immune system. The rejection for lack of enablement should also be withdrawn.

CONCLUSION

Applicants ask that all claims be examined and allowed. Attached is a marked-up version of the changes being made by the current amendment. No fee is believed due in connection with this Amendment. If there are any charges or credits, please apply them to Deposit Account No. 06-1050, referencing Attorney Docket No. 06558-004006.

Respectfully submitted,

Date: October 7, 2002

Lee Crews, Ph.D.
Reg. No. 43,567

Fish & Richardson P.C.
225 Franklin Street
Boston, Massachusetts 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

Version with Markings to Show Changes Made

In the Claims:

Claim 8 has been amended as follows:

--8. A method of inhibiting malignant [neoplastic] cell growth in a mammal at risk for malignant cell growth, the method comprising administering [nucleosomes] to the mammal [in] an amount of nucleosomes effective to elicit the production of sufficient antinuclear autoantibodies to inhibit malignant [neoplastic] cell growth in the mammal.--